

Assessment Protocol for Hairpin DNA Usability

Run a BLAST search on the two initiator sequences.

e.g.)

S41-H1 TGTTGCAAAGGAACGTCGAGCTGTAATGGTGCTCGACGTTCC

S41-H2 GCTCGACGTTCTTTTGCAACAGGAACGTCGAGCACCATTACA

S41-I1 GCTCGACGTTCTTTTGCAACA

S41-I2 TGTAATGGTGCTCGACGTTCC

At this time, it is ideal for the initiator sequence to be in the same direction and have a homology of 13 bases or fewer.

S41-I1 5'- **GCTCGACGTTCTTTTGCAACA** -3'

A certain mRNA 5'- AT**GCTCGACGTTCTTA**ACC-3'

↑This is not a problem.

Additionally, complementarity does not pose a problem.

A certain mRNA 5'- ATAGGAACGTCGAGCAACC-3'

In other words, when performing a BLAST search, if the homologous sequence shows a strand: plus/plus, it is desirable for the homology to be 13 bases or fewer. If the strand is plus/minus, it can be considered acceptable without issue.

Sequences with 14 or more bases of homology should be avoided as much as possible, but in some cases, they may still be acceptable.

S41-I1 [GCTCGACGTTCTTTG]CAACA

S41-I2 TGTAATGGTGGCTCGACGTTCC

It is acceptable to include the underlined stem region sequence; however, if the opposite toehold sequence contains five or more bases, there is a high likelihood that it may generate noise.

The genomic information can be ignored, as hybridization with DNA on the chromosomes will not occur unless special processing is performed.